1966

UT-AEC Agricultural Research Laboratory

University of Tennessee Agricultural Experiment Station

UT-AEC Agricultural Research Laboratory

Oak Ridge, Tennessee

THE AGRICULTURAL RESEARCH LABORATORY
operated by
THE UNIVERSITY OF TENNESSEE AGRICULTURAL EXPERIMENTAL STATION
for the
U. S. ATOMIC ENERGY COMMISSION
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THE COVER PHOTO shows the main laboratory and administrative area with Melton Hill Lake in the background. The original laboratory building formerly Scarboro School, is in the right foreground. The building has been renovated and an addition made to the right side.

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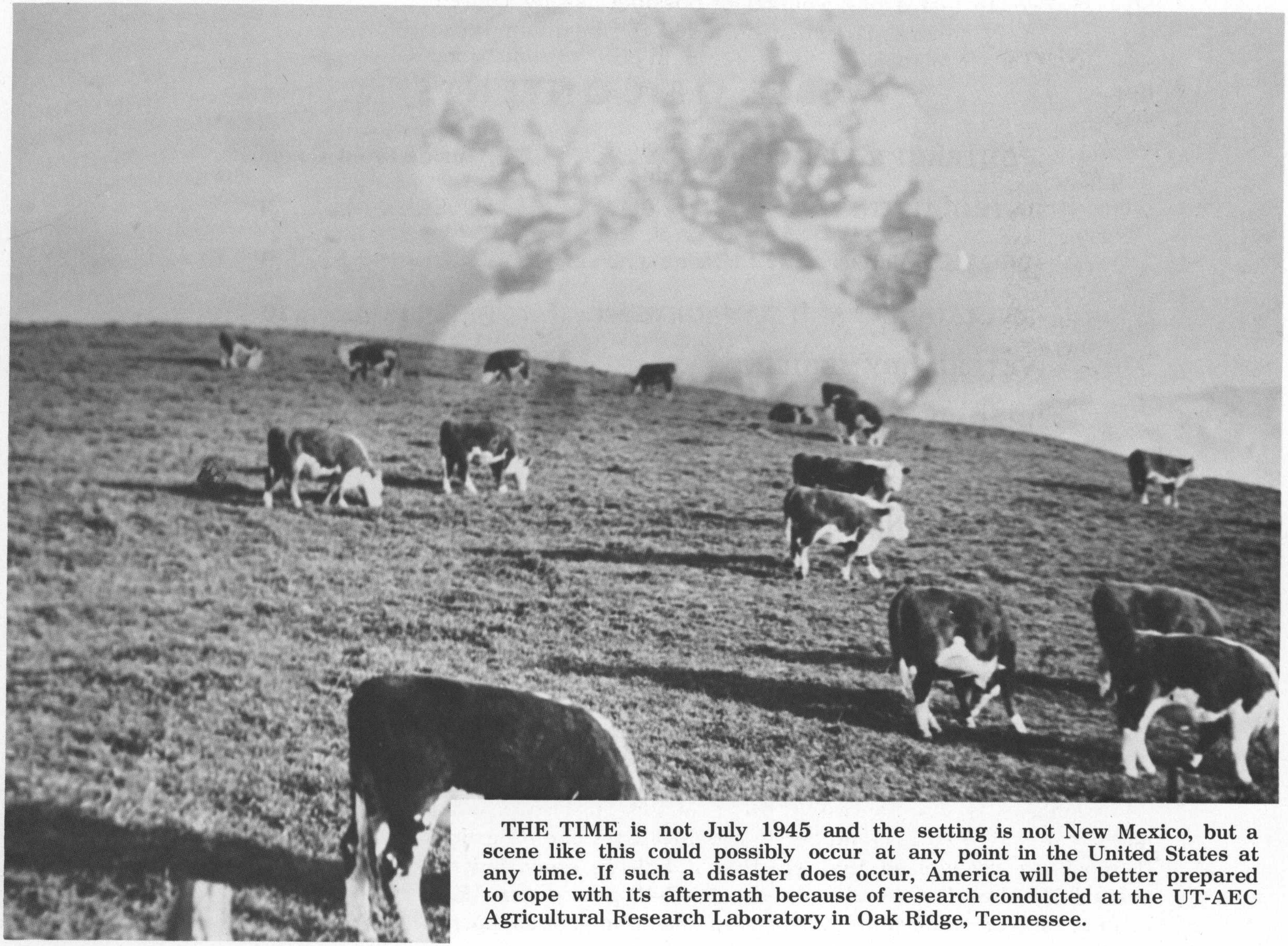
TABLE OF CONTENTS



THE TIME is not July 1945 and the setting is not New Mexico, but a scene like this could possibly occur at any point in the United States at any time. If such a disaster does occur, America will be better prepared to cope with its aftermath because of research conducted at the UT-AEC Agricultural Research Laboratory in Oak Ridge, Tennessee.

INTRODUCTION

In July of 1945 a group of scientists gathered at Alamogordo, New Mexico, to test a new weapon for possible use by United States military forces. That test heralded the beginning of a new era in warfare, in scientific research, in power production, in fact in many aspects of life throughout the world. The test was the explosion of the first atomic bomb.

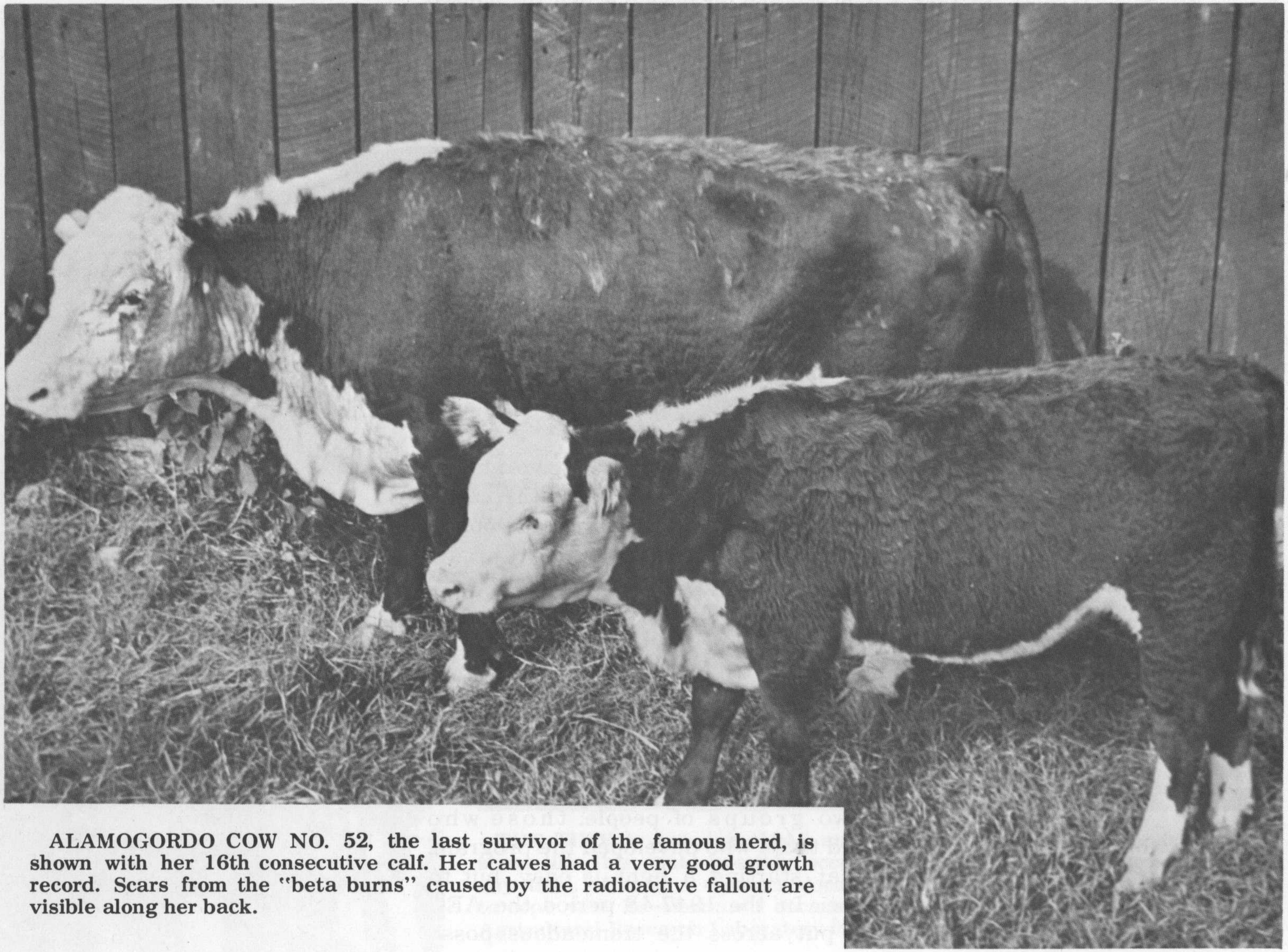
Nuclear scientists connected with the project may well have anticipated many of the events that would follow directly or indirectly from the bomb test, but certainly none of them could have known that a direct result of the test would lead eventually to the establishment of a laboratory specializing in agricultural research.

The detonation of the bomb kicked up a cloud of radioactive dust which settled over the area. Some of the dust fell on a herd of Hereford cattle grazing in the area. Soon the cattle developed open sores and lesions along the area of their backs where the dust had settled.

The owners of the cattle approached responsible governmental officials with the problem. The government bought the cattle and shipped some of them to Oak Ridge, Tennessee, for observation by the research staff of the Manhattan Project, predecessor of the Atomic Energy Commission.

Almost three years later, in May, 1948, these same cattle led the United States Atomic Energy Commission and the University of Tennessee to agree to cooperate in a research program in the general field of radioisotopes and radiation in agriculture. The result of that agreement is the University of Tennessee-Atomic Energy Commission Agricultural Research Laboratory.

Recognition is due to two groups of people: those who foresaw the possibilities, and those who developed the program. It must be recognized that starting a fight is easy, but to sustain it takes fortitude. In the 1947-48 period the AEC needed public support to put across the tremendous possi-



ALAMOGORDO COW NO. 52, the last survivor of the famous herd, is shown with her 16th consecutive calf. Her calves had a very good growth record. Scars from the "beta burns" caused by the radioactive fallout are visible along her back.

bilities to be exploited in the use of radiation and radioisotopes in scientific research. Agriculture and medicine were selected as the first fields in which the vast potential of the new developments was to be exploited. As it developed, the greatest progress for the first year or two was in agriculture.

It seems appropriate to mention a few names, and it is hoped that not too many of those who had important parts in the conception and development of the Laboratory have been overlooked. When the Commission and the University began discussing the project, Shields Warren was director of the Commission's Division of Biology and Medicine and Jim Jensen was the head man in biology. A.H. Holland and Ed McCrady were in the operations office of the Commission in Oak Ridge. F.H. Chance, director of the Tennessee Agricultural Experiment Station, and C.S. Hobbs, head of the UT Animal Husbandry Department were the University's chief representatives in the negotiations.

Unfortunately, most of these men have moved on into other fields. Dr. Hobbs remains as head of UT's Department of Animal Science, and Dr. Warren remains active in radiobiology. Dr. Chance has retired. Dr. Holland is executive vice president of Cortez F. Enloe Inc. Dr. Jensen is president of Oregon State University and Dr. McCrady is vice-chancellor and president of the University of the South.

The difficult task of developing a laboratory incorporating the ideas of all concerned was given to Cyril Comar, a young biochemist from Florida with large animal experience. He laid the framework and demonstrated how the laboratory could and should function. When Dr. Comar moved on to a position with the Oak Ridge Institute of Nuclear Studies, he was succeeded by Homer Patrick, a poultry nutritionist. Dr. Patrick left to become head of the Department of Biochemistry at West Virginia, and was succeeded as laboratory director in December 1957 by Nathan Hall, a soil scientist.

The Agricultural Research Laboratory operates in the precarious gap between fundamental research and applied science. It is believed that the role of large animal and whole plant research must be to bridge the gap. In this sense it is somewhat like an industrial pilot plant.

Originally the Project had one main objective—"The Investigation of the Effects of the 1945 Bomb Irradiation upon the General Health, Growth, Breeding Efficiency, and Relative Fertility of the Exposed Hereford Cattle and Their Offspring." Since its inception in 1948, the scope has changed to that of a laboratory devoted to the study of radioisotopes and radiation in agriculture.

A threefold objective was outlined for the Laboratory in the University of Tennessee Agricultural Experiment Station Annual Report of 1956. These objectives were: first, to carry on certain programmatic work requested by the Atomic Energy Commission; second, to carry on fundamental studies on agricultural problems using radioactive isotopes and radiation; and third, to enable graduate students and scientists to become acquainted with the application of nuclear energy in the field of agriculture. It is easily envisioned that these three objectives are so mutually interrelated that it is difficult to draw a line separating the activities.

The Laboratory conducts research on basic agriculture problems, on fundamental and practical problems associated with the entrance of fission products into the food chain, and on the effects of external irradiation on animals, plants, and seeds. The educational aspects of the program are associated with the research activities and limited to graduate students, postdoctoral employees and visiting professors. The staff also participates in the travel lecture program of the Oak Ridge Institute of Nuclear Studies.

The "Alamogordo" cattle arrived in Oak Ridge in December, 1945, and January, 1946. They were turned over to the Roane-

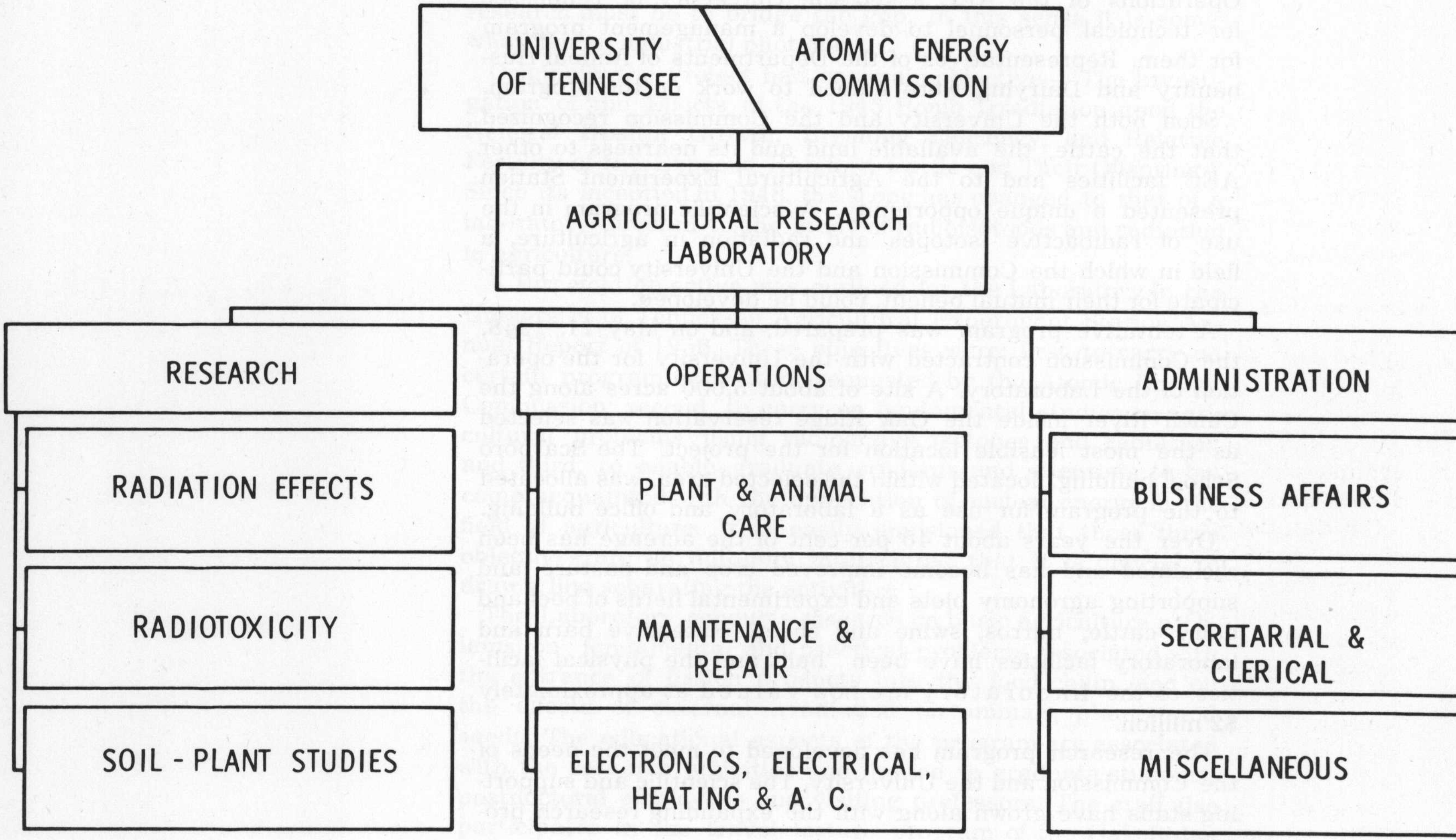
Anderson Company, an Oak Ridge operating contractor, and were maintained and observed until the Spring of 1948 when the chief of the Office of Research and Medicine for Oak Ridge Operations of the AEC asked the University of Tennessee for technical personnel to develop a management program for them. Representatives of the Departments of Animal Husbandry and Dairying were named to work on the program.

Soon both the University and the Commission recognized that the cattle, the available land and its nearness to other AEC facilities and to the Agricultural Experiment Station presented a unique opportunity. A scientific program in the use of radioactive isotopes and radiation in agriculture, a field in which the Commission and the University could participate for their mutual benefit, could be developed.

A tentative program was prepared, and on May 11, 1948, the Commission contracted with the University for the operation of the Laboratory. A site of about 5,000 acres along the Clinch River inside the Oak Ridge reservation was selected as the most feasible location for the project. The Scarboro School building, located within the selected area, was allocated to the program for use as a laboratory and office building.

Over the years about 40 per cent of the acreage has been reclaimed and has become improved crop and pasture land supporting agronomy plots and experimental herds of beef and dairy cattle, burros, swine and sheep. Extensive barn and laboratory facilities have been built and the physical facilities of the Laboratory are now valued at approximately $2 million.

The research program has developed to meet the needs of the Commission and the University. The scientific and supporting staffs have grown along with the expanding research program.



UNIVERSITY OF TENNESSEE
ATOMIC ENERGY COMMISSION
AGRICULTURAL RESEARCH LABORATORY
RESEARCH
OPERATIONS
ADMINISTRATION
RADIATION EFFECTS
RADIOTOXICITY
SOIL - PLANT STUDIES
PLANT & ANIMAL CARE
MAINTENANCE & REPAIR
ELECTRONICS, ELECTRICAL, HEATING & A. C.
BUSINESS AFFAIRS
SECRETARIAL & CLERICAL
MISCELLANEOUS

ORGANIZATION

The Laboratory, with its associated farm land, is an Atomic Energy Commission-owned installation operated by the University of Tennessee under contract. Funds for operating expenses and construction are provided annually by the Commission.

Currently the Laboratory has about 160 permanent employees. In addition to these, there are a variable number of visiting investigators and graduate students. Whenever possible, students are employed during the summer.

On the theory that the success of any organization depends on the competence and enthusiasm of the staff rather than on the form of the organizational chart, this laboratory has chosen a functional alignment. There are three major divisions. Administration includes all personnel involved in business affairs, secretarial and clerical services and a miscellaneous group. The research division is divided into three principal sections, and the operations division includes plant and animal care and the maintenance and repair of the physical plant and its equipment.

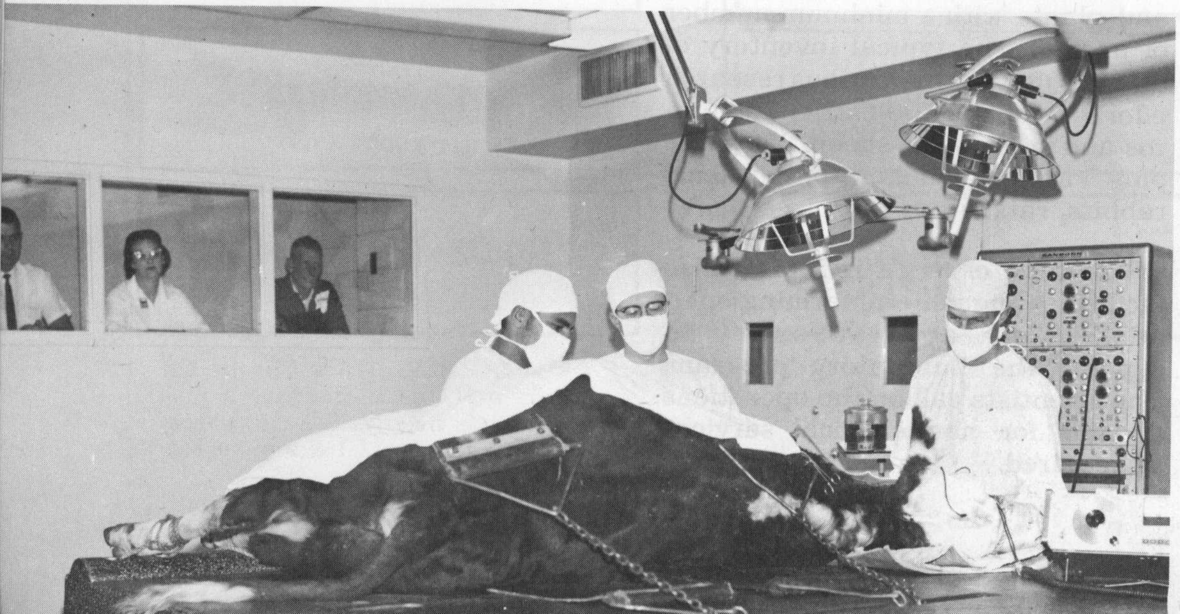
The director of the Tennessee Agricultural Experiment Station is the project leader and reports to the USAEC for the University. The laboratory director is in charge of the day-to-day operations of the Laboratory.

Probably the only unique feature of the organization is the fact that all animal and plant caretakers are in operations rather than being assigned to specific research areas. It is believed that this system assures the desired flexibility and permits the best care of the animals and plants with a minimum number of people. The typical inventory of animals used in the various research efforts is about 500 cattle, 300 burros and ponies, 250 sheep, 250 swine plus variable numbers of chickens, rabbits, rats, etc.

This type of organization also facilitates the general farming operations which are necessary to support the Laboratory program. The scientists call on the operations division for non-technical services as required.

ANIMAL CARE AND MANAGEMENT

SURGICAL PROCEDURES involving large animals are performed in this modern operating room. The operating table is hydraulically operated, and tilts to permit easier handling of the animal. The Hereford cow is under electro-anesthesia. The observation area in the background permits interested personnel to watch operations without interfering.



The day-to-day care of the herds and flocks is vital in providing a healthy and suitable group of animals for research purposes. Through the summer animals are run on pasture so they may forage for themselves as much as possible. About 2000 acres of pasture are used in this manner. In addition large quantities of hay, corn silage, and grass silage are harvested to provide forage in the winter. Mixed concentrates supplement the hay and silage in the animal rations.

Manpower to care for the animals involved in experiments and to maintain breeding herds and flocks comes from a pool of men assigned to crews. The men in each crew are responsible for the feeding and routine sanitation procedures required by the animals in their group.

If sickness develops among the animals the veterinarian in charge of herd health is contacted. Animals requiring only brief treatment are usually cared for in the area in which they are normally kept. Animals requiring long-term medical care or extensive surgery are removed to the hospital barn for care and treatment.

The hospital barn provides individual pens for confining animals during treatment. A team of men provides care of animals confined here and also maintains the surgery building. The surgery building has two operating rooms, one designed for work with animals weighing approximately 200 pounds or more. It has a hydraulically-operated table for restraining large animals during surgery. The other unit is designed for small animal surgery. In addition, there is a recovery area, a clinical laboratory and working area for the research staff.

The veterinarian in charge of herd health keeps abreast of disease problems of the herds and flocks and controls them through appropriate medication, vaccination or other forms of therapy. He is responsible for treatment of the animals confined in the hospital barn and coordinates with various project leaders to insure that treatment does not interfere with ongoing research efforts.

VETERINARY MEDICINE

The Laboratory's research in veterinary medicine deals primarily with the clinical aspects of both early and late effects due to irradiation injury. The early effects studies are concerned with problems of acute irradiation injury related to survival of animals used for food. The principal objective of the late effects studies is to ascertain, by clinical investigation, indications of delayed damage to animals surviving exposure to gamma and/or neutron radiation.

Lethality has been the most commonly used end-point for acute radiation effects. The $LD_{50/30}$ (lethal dose for 50 per cent of the exposed animals within 30 days) has been established for cattle, sheep, swine, and burros. There are many variables which will alter the response to irradiation. There are also differ- of animals to a radiation dose; thus, the $LD_{50/30}$ doses measured are only approximations. For example, the dose-rate (radiation intensity), type of radiation, and quality of radiation are variables which will change the biological response to irradiation. There are also differences in response among species, breeds, and lines or strains within breeds.

One phase of the early effects studies is concerned with the effects or radiation on the central nervous system (CNS). Signs of CNS derangement can be induced in burros with low doses, either whole-body or to the head only, with either gamma or neutron radiation if administered at a high dose-rate. This phenomenon does not occur in cattle,

A VETERINARIAN checks the mouth and teeth of a burro foal. All animals used at the Laboratory get regular physical examinations.

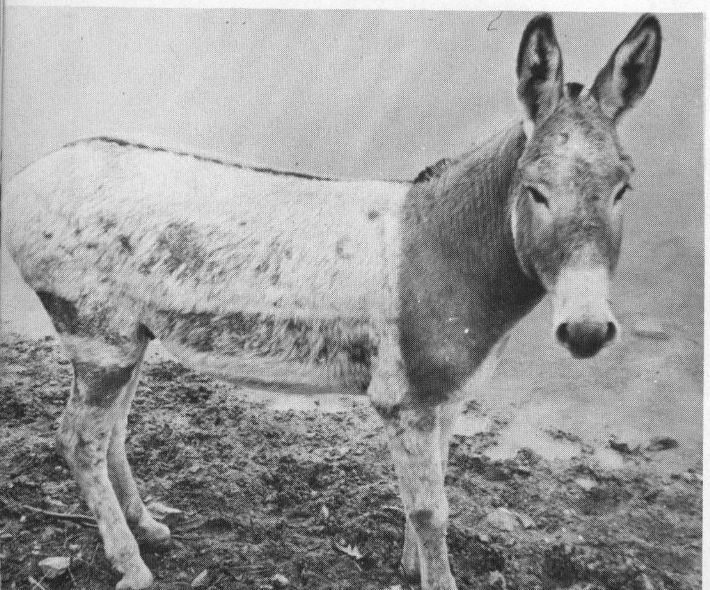
sheep, or swine. Burros and sheep are used for comparative studies of the physiology of the CNS before and after radiation. The change in composition of the cerebrospinal fluid, the movement of electrolytes, protein, etc., across the blood-strain barrier, the utilization of oxygen by the brain tissue, and the ability of the nervous system to respond to drug stimuli are the principal parameters. Shielding of different parts of the CNS during irradiation is a technique used to evaluate response.

The acute response of all large animal species appears to be altered by dose-rate. An investigation of this is planned following the construction of a new Co^{60} -irradiation facility which will permit exposures with dose-rates from 0.5 to 50 r/minute. Swine have been selected for this initial study.

A TECHNICIAN takes a blood sample from a hog which has been exposed to radiation. Hematological data is an important part of the medical history of experimental animals.

Approximately 450 cattle, swine, and burros which have been exposed to gamma radiation and mixed neutron-gamma radiation from an atomic detonation are surviving doses ranging from 150 to 700 rads. These animals have been assigned for study throughout the remainder of their life span. Many of the animals were irradiated in the early 1950's.

These animals are checked each day and are subjected to various clinical procedures and measurements on either a quarterly or semi-annual basis. Emphasis is on longevity and detection of physiological



PARTIAL SHIELDING produced the striking contrast in hair color shown by this burro. The animal's head and neck were shielded during a radiation exposure while the posterior portion of its body was unshielded. This is part of a study of central nervous system derangement in the burro.

BURROS IRRADIATED at an early age are fed controlled rations varying only in the level of protein to check the effect of diatary variations on long term radiation responses.

PONIES ARE BEING TRAINED to perform certain tasks in preparation for a study of the effects of radiation on an animal's ability to work. Some of the ponies will be irradiated at various dose levels, and their subsequent efficiency compared to that of control animals.

changes which may be attributed to irradiation.

Another phase of this study is the evaluation of growth and nutrition requirements of burro foals exposed to single doses of Co^{60} -gamma radiation at an early age. After weaning at six months of age, the animals are fed a controlled diet, varying only in level of protein, for a period of 12 months. The principal items studied are growth rate, feed utilization, hematology, blood enzymes, and thyroid function. Apparently if a young animal (burro) absorbs a moderate (approximately 200 rad) amount of gamma radiation, and is placed on an adequate to good protein-level diet, no immediate ill effects of the irradiation are observed after the initial recovery period. Preliminary results indicate that inadequate dietary protein levels do not compound radiation effects to the degree expected.

Does exposure to sublethal doses of radiation impair the work capacity of an animal after apparent recovery from the initial or early effects? This question is being considered in a new study with Shetland ponies. The ponies are assigned specific amounts of work to be performed within a predetermined time. Various physical and physiological tests are made periodically to evaluate the physical capability of each animal.

DIAGNOSTIC PATHOLOGY

Research in pathology has consisted principally of the gross and microscopic examination of tissues from four species of large domestic animals subjected to ionizing radiation from various sources. Salient lesions observed have included the following: disseminated hemmorrhages; ulcers in the alimentary canal; atrophy or necrosis of the lymphatics and bone marrow; encephalitis; uterine, hepatic, and intestinal neoplasia; and extensive areas of necrosis in the skin. The histopathological evaluation consists primarily of perusal of tissue sections with the aid of the conventional microscope and the usual hematoxylin and eosin stain. Special histochemistry is employed when necessary.

There is also intermittent diagnostic work associated with animals not yet directly involved in research with ionizing radiation. Gross and microscopic study of tissues from smaller laboratory animals utilized in radiation research is conducted. Bacteriology is routinely utilized to determine the presence of primary and secondary infections with microorganisms.

To date no lesion has been observed that is unique to irradiated animals. However, certain lesions apparently occur with increased frequency in long term survivors.

Pathologists hope that with the accumulation of information we will be able to predict the diseases leading to terminal illness in animals surviving relatively large doses of ionizing radiation.

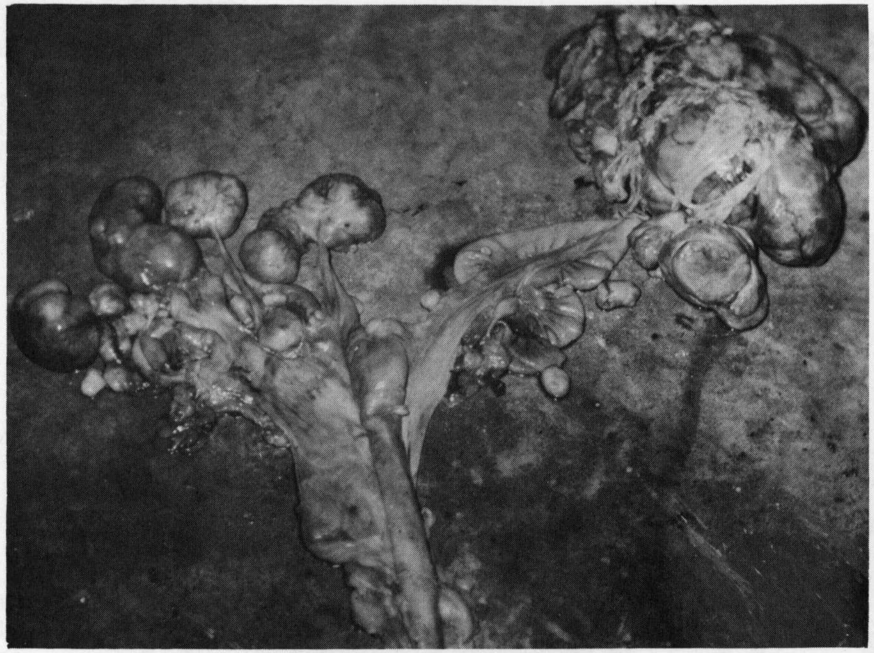
In this connection we have noted that cadavers of swine subjected to various doses of total-body irradiation from an atomic bomb at the Nevada Test Site in 1957 displayed a significant number of neoplasms at three principal locations.

The uteri were often affected with pedunculated and intramural tumors derived primarily from the smooth muscle of the uterine wall. These uterine tumors were comparable to the uterine "fibroids" noted frequently in women during the reproductive years. The livers of a number of these swine contained rather sharply delimited hepatomas. Annular strictures in the form of apparent sclerosing adenocarcinomas caused partial blockage in the small intestines of several of these animals. The neoplastic tissues seemed consistently to remain localized, although in many cases they obviously contributed to impairment of the functions of the affected organs.

The controls have not thus far shown comparable lesions.

In the course of experiments in which pigs were exposed to unmodulated fast neutrons, dramatic skin lesions were developed. In order to further understand the effects of neutron irradiation on the skin, white pigs have been anesthetized and exposed to 400-450 rads of neutrons delivered as a collimated beam anesthetized and exposed to 400-450 rads of neutrons delivered as a collimated beam approximately two inches in diameter. All other parts of the animals were well shielded. After exposure, these swine were observed carefully for skin changes attributable to the effects of irradiation, and the affected skin areas subjected to thorough gross and microscopic examination. Aside from mild transient erythema that diminished rapidly between one and seven days, no tissue changes attributable to irradiation were observed in this group of animals. Two additional white pigs were subsequently exposed to the neutron beam under identical circumstances. These animals have been studied for five months, and periodic observations are continuing. Equivocal erythema and edema were observed at the sites of irradiation on these animals throughout the first week after exposure. Distinct areas of erythema were first observed during the second week after irradiation and these changes persisted for the next thirty days. They then gradually faded during the remainder of the first two months after irradiation. Four months after irradiation the only recognizable skin alteration was a barely perceptible area of light tan discoloration.

This is contrasted to skin lesions observed in whole-body-irradiated swine which failed to heal over a period of six to eight months.



SHOWN ARE multiple pedunculated and intramural leiomyomas of the uterine wall in a swine from a group exposed to mixed gamma-neutron radiation from detonation of an atomic bomb at the Nevada Test Site in the spring of 1957.

REPRODUCTIVE PHYSIOLOGY

A study of radiation effects on lifetime reproduction has been initiated to determine the effects of varying levels of acute total-body irradiation on (1) fertility and longevity of beef cows, (2) viability and growth performance of their calves, and (3) any long-term pathological response of the cows. Sexually mature females (18 to 20 months of age) of the Hereford breed were exposed to varying doses up to 400 r in a single exposure and 600 r in a fractionated double exposure.

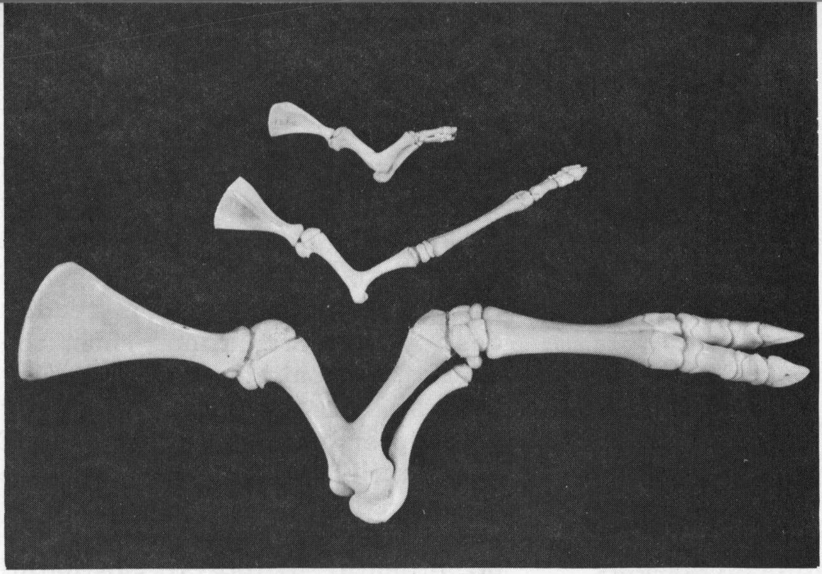
Approximately 200 survivors and controls are being maintained for their reproductive life and a more valid evaluation can be made of the effects of radiation on reproduction and longevity. Performance of the offspring from these survivors is determined up to weaning and, in most instances, through a post-weaning feeding period. Records of regular physical examinations including blood counts and eye examinations are kept on all survivors. Clinical records are maintained on all such animals and postmortem examinations are made by a veterinary pathologist.

Radiation has been demonstrated to be quite detrimental to the developing embryo, certain embryonic systems being susceptible to damage during specific periods of development. An example of such damage is illustrated showing the same fusion of the radio-humeral joint as a result of exposure on the 21st day of gestation in swine, the 23rd day in sheep, and on the 32nd day in cattle. Such species comparisons aid in predicting the extent of fetal damage to be expected when the pregnant female is subjected to irradiation.

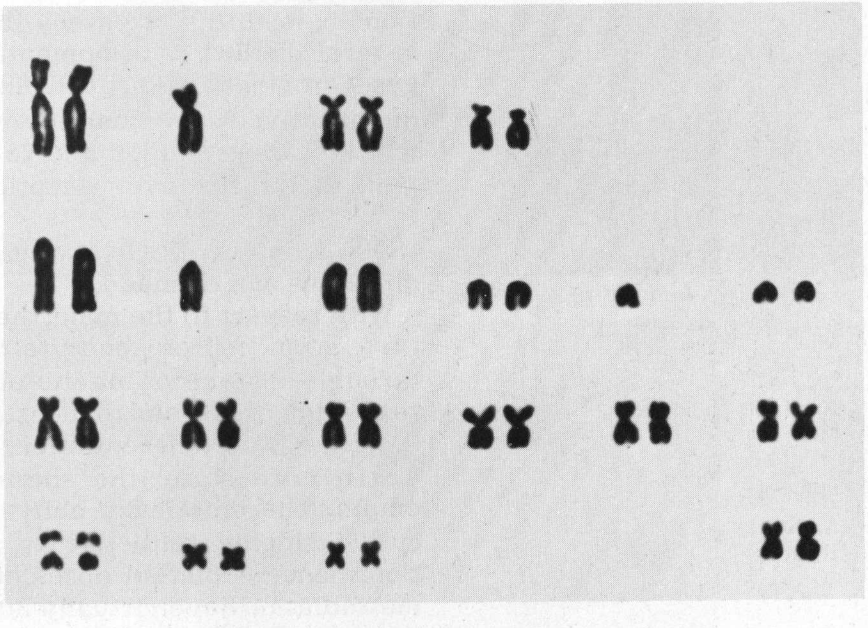
Dose-level studies during certain sensitive periods of gestation are being used to establish the dose dependence and to predict the probabilities of an expected event. The relative efficiencies of gamma and neutron irradiations for embryo damage are also being compared.

Efforts to define the mechanisms involved in such damage are concentrated on the growth in artificial media of tissue from sensitive regions of the embryo. The behavior of these tissues following irradiation is examined from the standpoint of induced chromosomal abnormalities and changes in the over-all viability of cells in the system.

Techniques have been developed for the successful in vitro growth of white blood cells from the major domestic species and for the direct visual examination of their chromosomes. Present applications of this method are designed to elucidate some of the differences between cells from various species in their res-



PRENATAL IRRADIATION on the 21st day of gestation in swine, top, 23rd day in sheep, center, and 32nd day in cattle, bottom, produced fusion of the radio-humeral joint in the developing fetus. This type of damage is very specific for certain periods of embryonic development.



THIS KARYOTYPE is from a 37-chromosome European wild pig. Note the three unpaired chromosomes. Some wild pigs have 36 chromosomes, while all domestic breeds have 38.

ponse to irradiation both inside and outside the animal. The same methodology is being applied in an attempt to find a cytological basis for the variation in radiosensitivity which exists between individuals of a species and, if possible, to predict such sensitivity.

These culture techniques are proving to be a valuable aid in the study of genetic problems, especially the case of chromosome inheritance patterns in European wild pigs which are unusual in that their chromosome number shows variation between individuals.

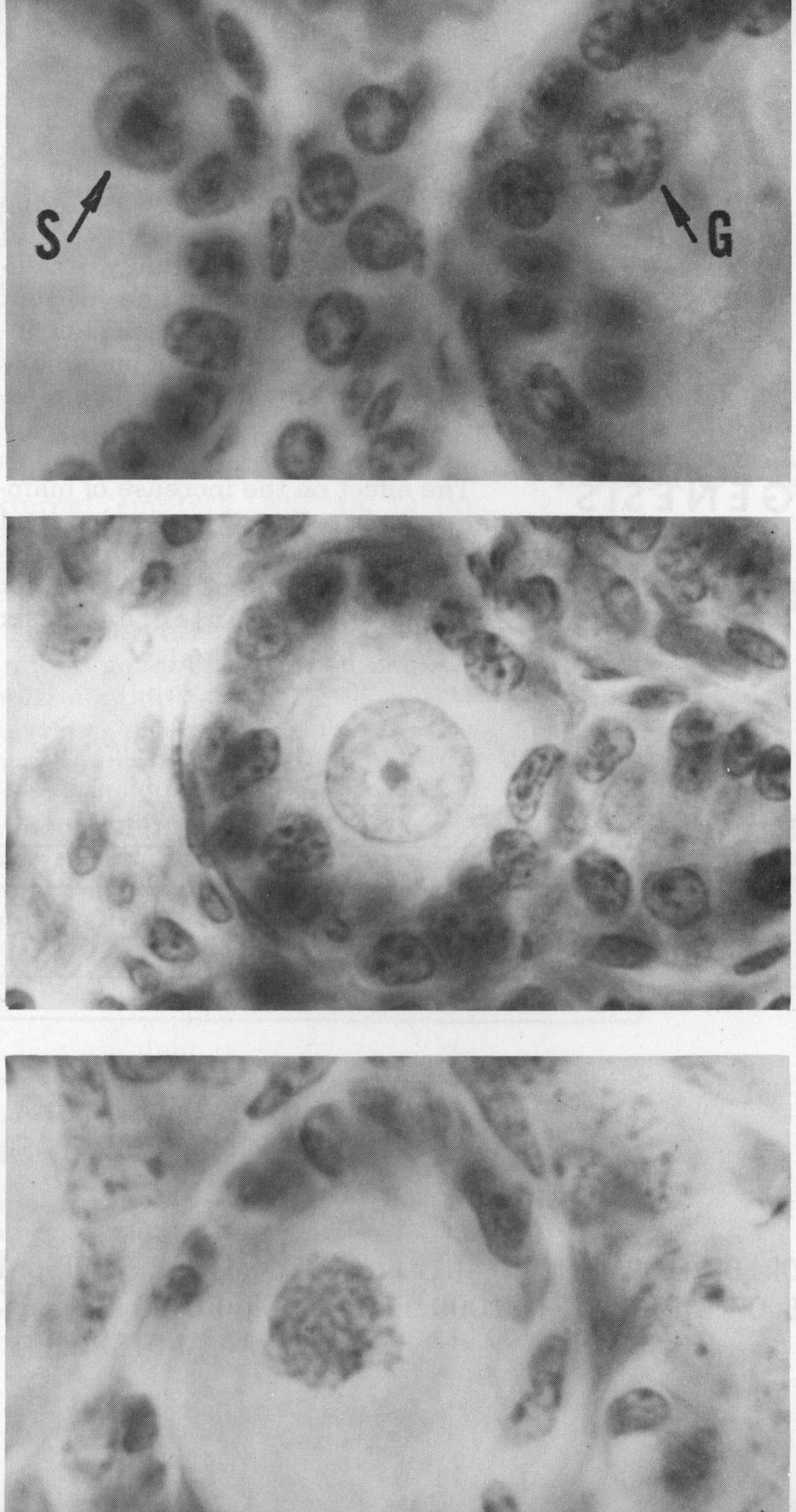
The mammalian germ cell from its inception to its death or utilization in fertilization passes through several distinct developmental stages. Our objectives are to define both quantitatively and qualitatively the limits of these stages and to determine their respective susceptibilities to radiation.

As examples, the following generalizations can be made:

With respect to the male, the primitive germ cell or gonocyte passes through a stage of mitotic activity to a stage of rest and thence through a process of differentiation to its definitive state, the spermatogonium. The mitotically active gonocyte is highly refractive to radiation; hence sublethal doses of gamma-radiation have virtually no effect on the fertility of boars and bulls irradiated prior to days 50 and 80 of embryonic development, respectively. In contrast to the foregoing, the "resting" gonocyte is readily destroyed by radiation. Thus, the sperm producing capability of bulls irradiated at any age between day 80 of prenatal life and day 80 of postnatal life is reduced by at least 50 per cent. The boar is similarly affected during the interval of day 50 prenatal to day 70 postnatal. By day 70 for the boar and day 80 for the bull of postnatal life, a majority of the primitive germ cells (gonocytes) have become spermatogonia and with this, other than the brief period of infertility which follows irradiation, superlethal levels of ionizing radiation are again required to induce sterility in the male.

In contrast to the male, where the transition from primitive germ-cell to spermatogonium occurs post natally, the primitive germ-cell of the female gives rise to the oogonium and the oogonium to the oocyte early in the period of fetal development (day 40, pig, day 75, ox). Germ-cells are generated throughout the life span of the male, but oogonial mitosis is discontinued during the prenatal state and the number of germ-cells available to the female is consequently fixed. Oocytes enter meiotic prophase and advance to its latter stages where they "rest", and no further chromosomal changes occur until either the beginning of follicle formation or ovulation, depending on the species in question. Hence, again depending on species, an oocyte can remain at one stage of development for days or years.

Each stage of the meiotic prophase differs in its susceptibility to radiation; thus, the amount of radiation necessary to sterilize the mammalian female varies widely with species as well as with the stage in germinal evolution. Between 100 and 200 roentgens of gamma-radiation will destroy the oocyte of the rat in its stage of rest (dictyate, a post-diplotene condition) and 400 to 500 and 600 to 900 roentgens are required to kill the 'resting' oocytes of the sow and cow. A chromosomal condition representative of diplotene characterizes the 'resting' oocyte of the sow and at a like state, the pachytene oocyte typifies the cow.

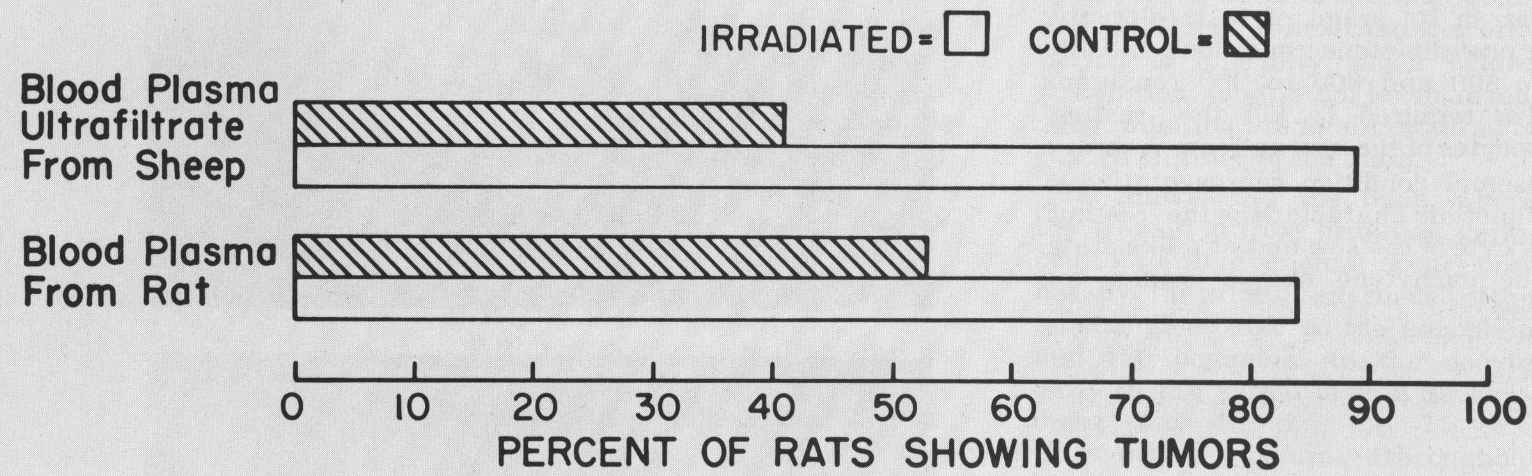


THE MORPHOLOGY of the gonocyte (G) and spermatogonium (S), as they appear in a bull testis aged 90 days, is depicted in the top photo. The center photo shows the "resting" oocyte of the rat, and the lower photo illustrates the morphology of the "resting" oocyte of the ox.

A higher incidence of mammary tumors was found in the rat when injected at the time of birth with blood plasma from lethally irradiated sheep. Fractionation of that plasma resulted in a significant increase in the incidence of mammary tumors after injection of blood plasma ultrafiltrate.

TUMORGENESIS

The effect on the increase of mammary tumors was only observed when rats were injected during the early days following birth and not when they were injected at six months of age. The increased incidence was hormonal dependent since it was found only in intact females. Rats in which the ovaries were extiriated shortly after birth and which were then injected with plasma ultrafiltrate showed no change in the normal incidence of such tumors. Most of the tumors observed were benign mammary tumors. So, as is found with whole-body irradiation, either blood plasma or its ultrafiltrate from irradiated animals increased the incidence of mammary tumors in the rats in our colony.



TUMOR DEVELOPMENT IN SURVIVING FEMALE SPRAGUE-DAWLEY RAT AFTER INJECTION AT THE TIME OF BIRTH WITH BLOOD PLASMA OR BLOOD PLASMA ULTRAFILTRATE FROM IRRADIATED RAT AND SHEEP

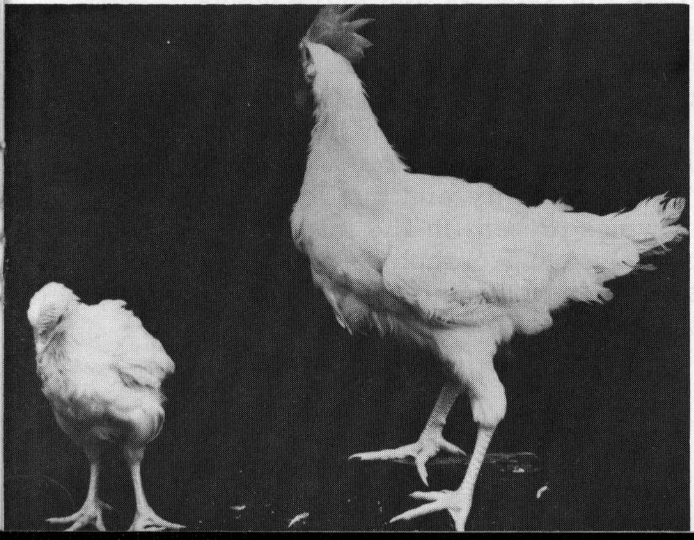
RADIOTOXICITY AND METABOLISM

Distribution and biological half-lives of fission products in particular tissues must be known in order to predict the hazards to be encountered from fission product contamination of the biosphere. The principal objective is to study the metabolism of fission products with specific reference to their role in the metabolic pathways and to determine the interaction of chemically similar nuclides.

The mechanisms of discrimination between elements at the interfaces of organs, cells and cellular particulates during the vital life processes of absorption, excretion, anabolism, catabolism, and reproduction are being investigated. The influence of dietary additives, hormones, metabolites, and antimetabolites on fission product metabolism is being studied to determine the possibilities of changing their metabolic pathways.

Cell fractionations are being performed to determine if differences exist in compartmentalization with time-post-contamination in an effort to assess radiodamage to individual metabolic sequences. This information will assist in the determination of the organ and cellular irradiation from internal emitters which, coupled with a knowledge of the effects of whole-body irradiation from external emitters, will aid in the development of safeguards to counter each exposure to radiation.

The embryo is the stage of development generally most susceptible to irradiation damage and should, therefore, provide a good index of this damage. The avian embryo is completely divorced from maternal influence during incubation, has a short incubation period, is inexpensive and is well suited for a study of this nature.



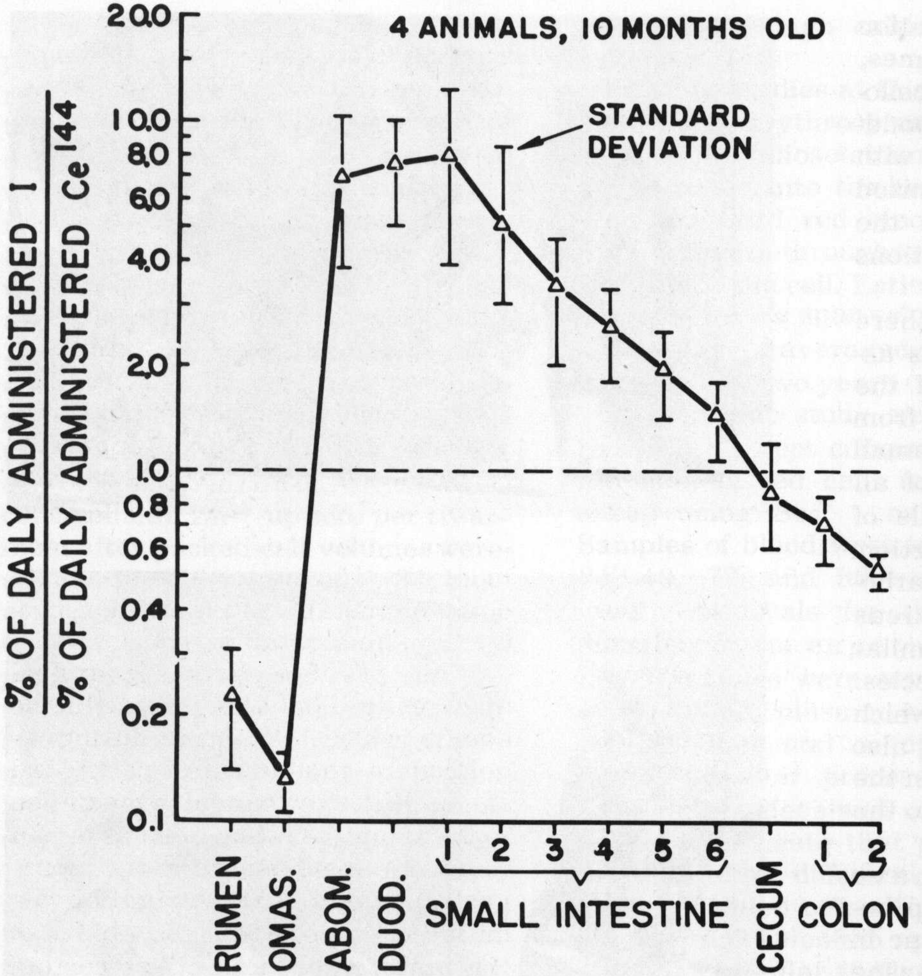
THE EGG FROM which the cockerel on the left was hatched was injected with 50 microcuries of radioactive iodine on the 14th day of incubation. The cockerel on the right was hatched from a control egg. Both are 10 weeks old.

The effects of gamma and neutron irradiation from external sources and alpha, beta, and gamma irradiation from internal sources upon embryos and birds and their subsequent growth and reproduction are being considered. In addition, the influence of the chemical form, specific activity, type and energy of the emission, half-life, and dose level of the radionuclide and its absorption by the various cells and organs are being studied in embryos and birds varying in age.

Iodine is both an essential element of nutrition and a fission product. Intake levels of radioiodine likely to cause observable injury to cattle are considerably greater than the levels required to render animal products unfit for human use. However, the isotope rapidly disappears from an animal's system due to excretion and physical decay. It is, therefore, of interest to determine the effects of radioiodine exposure on later production and health of the animals. In addition, altered iodine metabolism resulting from toxic levels of I^{131} should be investigated.

This can be accomplished using another radioisotope of iodine, I^{125} as a tracer before, during and after I^{131} administration. Developmental stages ranging from the unborn fetus to mature animals should be investigated. To date, four mature cows, three of which were pregnant, and six growing heifers have been fed single doses of 200 microcuries of I^{131} per kg body weight after preliminary measurements of thyroid uptake, thyroid secretion rate, and metabolism of daily tracer levels of I^{125}. The three cows, each of which received 100 millicuries of I^{131}, calved 15, 123 and 136 days after dosing. The 15-day calf was a cretin with no detectable thyroid activity. The 123-day calf died just after birth, while the 136-day calf was apparently normal. The cows, surviving calves, and the six heifers with their identical twin controls are being maintained for future measurements of iodine metabolism, growth, reproduction, and lactation.

In the past few years a great deal of investigation has dealt with the events which take place during digestion in the rumen of cattle and sheep. Digestion caudal to the rumen in these animals has been assumed to be the same as that in the monogastric animal. An investigation was initiated to ascertain the ability of the mature sheep to utilize certain carbohydrates in the small intestine. This ability depends on two things: (1) the quantity and quality of the enzymes present in the intestine to hydrolyze the carbohydrates to an absorbable form and (2) the absorptive ability of the intestine.



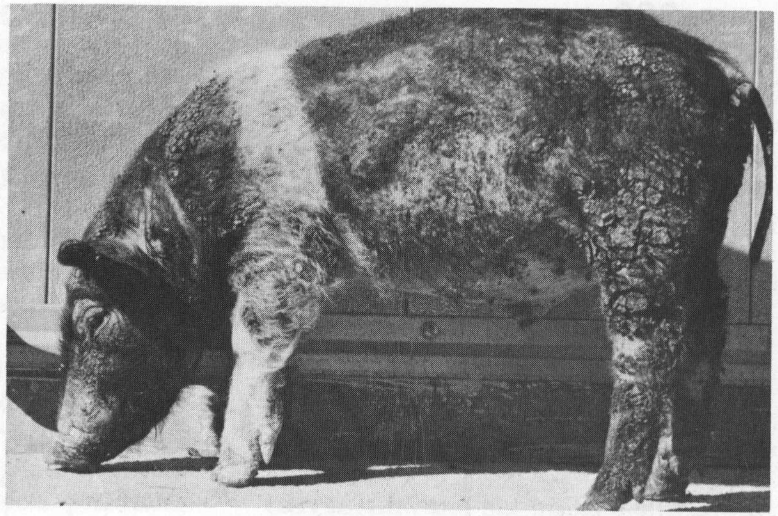
ABSORPTION AND SECRETION of radioiodine in the gastrointestinal tract of young cattle. A ratio of cerium (non-absorbed marker) and radioiodine of 1.0 means no net absorption or secretion. Iodine is absorbed from the rumen, resecreted to a high concentration in the abomasum, and reabsorbed in the lower tract. It is significant that two major absorption sites for iodine exist in the digestive tracts of cattle.

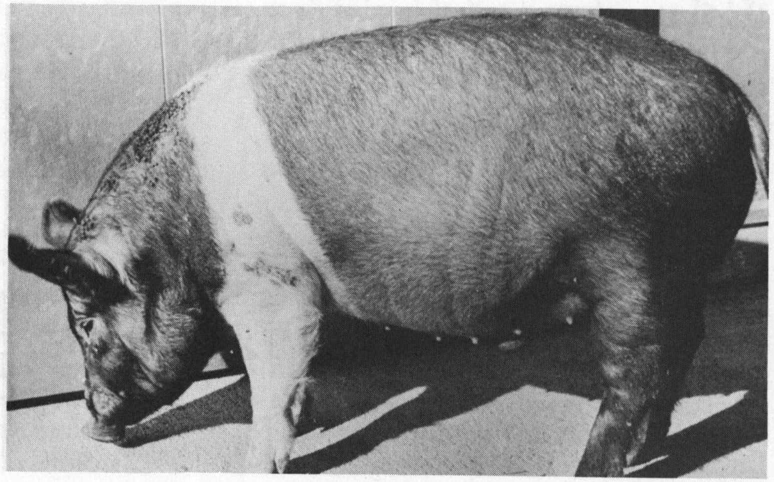
The first part of the investigation is the analysis for the enzymes, maltase, lactase, sucrase, cellobiase, and amylase. The corresponding substrates were incubated with saline extracts from homogenized intestinal mucosal cells from the duodenal, jejunal, and ileal sections of the small intestine.

Results to date indicate that there is more activity of all enzymes assayed in the mucosal cells of the jejunum or middle portion than from the other segments of the small intestine. The least amount of all enzyme activity is in the cells of the duodenal segment or first portion. These results for the relative carbohydrase activities in the three areas of the small intestine are similar to results obtained in other species.

Enzymes from the pancreas, which will be assayed at a later date, also empty into the small intestine in the duodenal area and contribute to the hydrolysis of carbohydrates.

Numerous investigators have demonstrated a relationship between dietary calcium and zinc in animals. A deficiency of zinc in swine rations produces a dematitis known as parakeratosis. Elevated levels of calcium tend to increase the incidence and severity of the disease, while supplemental zinc alleviates the condition or prevents it from occurring.





A HIGH-CALCIUM-LOW-ZINC ration produced the severe parakeratosis in the pig above after 16 weeks. The same animal, below, showed marked improvement after nine weeks on the same ration supplemented with 71ppm zinc.

In an attempt to investigate this problem further, 24 weanling barrows averaging 19 kg were allotted to six lots and fed either variable calcium, zinc, or fat levels for 16 weeks to determine effects on Zn^{65} retention by pig blood cells **in vitro**. Five ml of freshly drawn whole blood were obtained at two-week intervals and dosed with 200 microcuries of Zn^{65}. After incubation under O_2 atmosphere at 38° C for a two-hour period, the cells were separated from plasma and their radioactive zinc content determined. Average per cent cellular Zn^{65} uptake per treatment after packed-cell volumes were adjusted to 40 were: basal, 34; basal plus 71 ppm Zn, 26; basal plus 0.6 per cent Ca, 55; basal plus 71 ppm Zn plus 0.6 per cent Ca, 28; basal plus five per cent oil, 34; basal plus 0.6 per cent Ca plus five per cent oil, 51. Dietary calcium increased while dietary zinc decreased **in vitro** uptake of Zn^{65} throughout the experiment. Added oil exerted no significant effect on Zn^{65} uptake **in vitro**. Dietary treatment had no detectable effect on the stable zinc content of these cells. The effect of treatment upon the **in vitro** uptake of Se^{75}, Cs^{137}, I^{131}, Co^{60}, Mn^{54}, Fe^{59}, Cd^{115}, Cu^{64} by swine blood cells was also investigated with variable results. Four incidents of parakeratosis were observed in the eight pigs fed the high calcium-low zinc rations.

Previous studies at this laboratory with sheep erythrocytes have indicated that radioselenium (Se^{75}) is incorporated into the hemoglobin of newly-formed red blood cells and remains there throughout the entire life span of the cell. Estimates of the erythrocyte life span calculated from Se^{75} turnover averaged 157 days in sheep one to two years of age.

The present study was designed to make a direct comparison of the life span of red cells in swine and sheep using Se^{75} as a marker. Samples of blood were taken at six, 24, 48, 72, and 96 hours and at weekly intervals for the 161-day duration of the experiment. The blood samples were separated into whole blood, plasma, and packed-cell fractions and counted directly without further processing on an auto-gamma deep-well counter.

Results indicate that pig erythrocytes reacted differently to Se^{75} than did sheep cells. The initial uptake of radioselenium appears to be somewhat similar in the red cells of both pigs and sheep. However, the selenium did not remain at a near-constant concentration in the pig cells long enough to give an accurate determination of red cell life span. The selenium concentration remained relatively constant for a


PERKIN ELMER
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GRADUATE STUDENTS use an atomic flame spectrophotometer in the study of animal metabolism of radionuclides. Students sometimes develop new research techniques to help them solve problems encountered in studying the relatively new field of radioisotopes and radiation in agriculture.

period of time after initial incorporation into the sheep cells, and decreased rapidly to suggest a completion of erythrocyte life span.

Experiments are being prepared to study the mechanisms of incorporation, release, and utilization of Se^{75} in both sheep and swine erythrocytes in reference to red cell life span studies.

As a part of a study of differential tissue uptake of certain minerals in irradiated animals, hair and skin were collected from different species, washed and ashed for analysis of zinc and copper by atomic flame spectrophotometry.

The results showed that copper concentration in irradiated hair was higher than in nonirradiated hair. The copper content in the hair of different species was also considered. In swine, the red pigmented hair apparently shows the greatest concentration of copper with 16 ppm. In bovine the black hair shows six ppm, and in equine the light brown hair has the greatest concentration with eight ppm.

Studies indicated a dramatic increase in hair zinc content in unilaterally irradiated swine. Animals exposed to 742 rads demonstrated the highest content of zinc in irradiated hair with approximately 130 ppm. The high zinc concentration in the irradiated hair in swine is presumably based on the utilization of zinc in tissue repair.

In vitro uptake of Zn^{65} by red blood cells of equine exposed to 300 rads and swine exposed to 700 rads of neutron radiation was greater than for red blood cells from control animals.

The study also seems to indicate that zinc is selectively retained in the hair when hair and skin are compared.

Studies have been initiated to check metabolism of dietary zinc in neutron-irradiated swine.

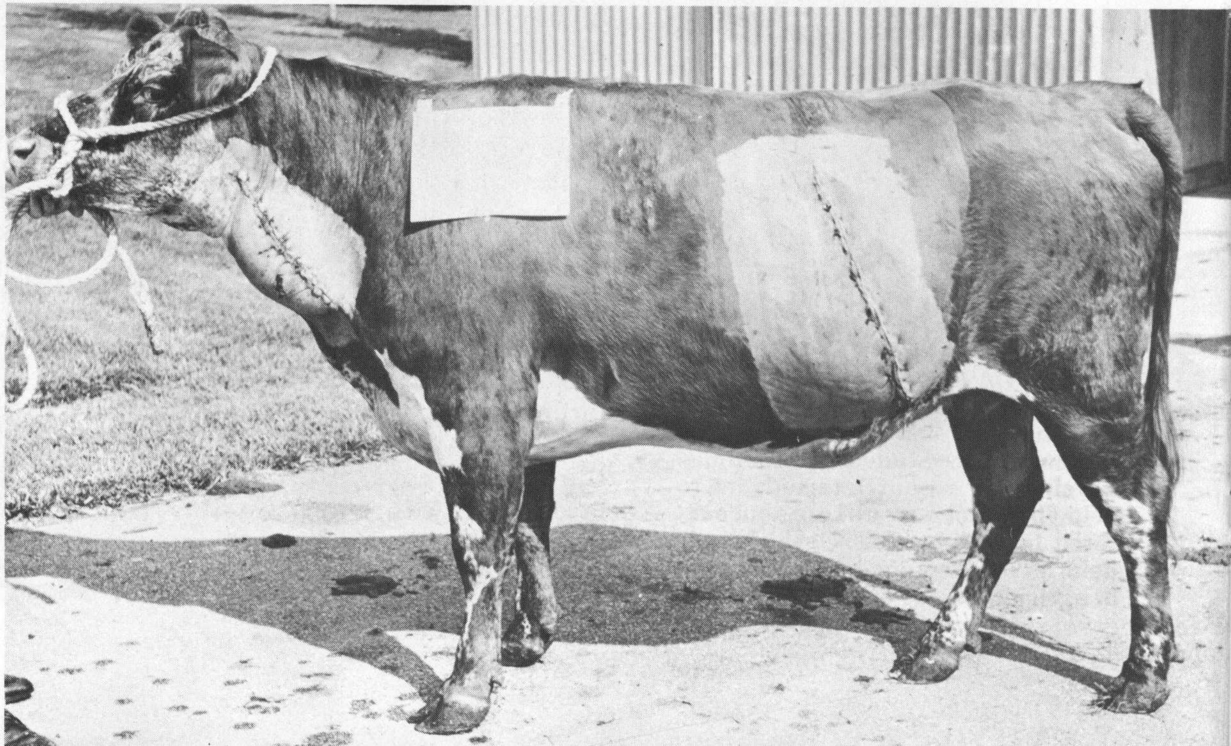
Fraternal twin calves are, in many cases, tolerant to the transplantation of tissues from one twin to the other even though they are genetically unlike. This characteristic is called "acquired tolerance" and results from anastomosis of the blood vessels of the two chorions during embryogenesis. Thus, a common blood circulation between the two embryos allows the migration and subsequent implantation of those cells which in later life cause tissue tolerance.

This phenomenon and how to produce it at will appears to have significance for the future of organ and tissue transplantation. Irradiation and drug treatments have been given to twin cattle to elucidate mechanisms for tissue compatibility.

Skin grafts and kidney transplants between twins have been made. Skin grafts from a co-twin in 10 cases have remained intact for two years and are still being retained without the use of immune depressants. Kidney transplants under the same circumstances remain intact and functional for at least several months, and there is no reason to believe that the kidney transplants will remain intact for less time than skin grafts. Emphasis in this work is shifting toward embryonic development and ways of inducing acquired tolerance.

This work is being done in cooperation with a University of Wisconsin geneticist.

A KIDNEY WAS REMOVED from its normal location (long incision on side) and transplanted to the neck of this cow's twin. A kidney from the twin was put in this cow's neck. The transplanted kidneys were functioning as this was written. The experiment is part of a study in which scientists are learning more about tissue tolerance and ways to induce it in animals.



FISSION PRODUCT CHEMISTRY OF SOILS

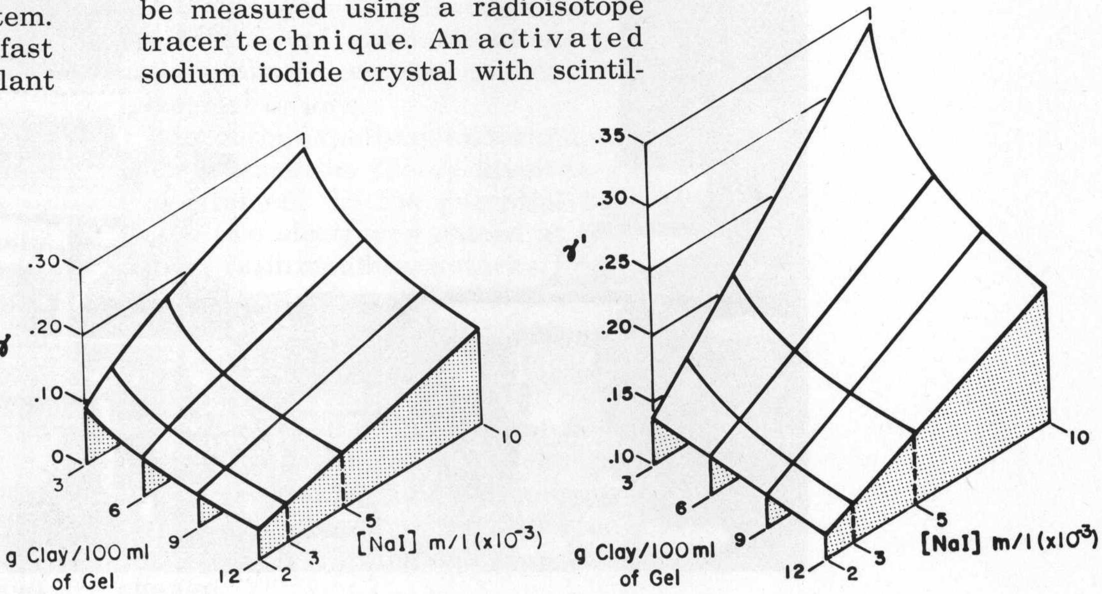
The objective of the soil chemistry research program is to study the phenomena affecting the entrance into the food chain of those fission products present at low levels and mixed with the soil. It has been assumed that the availability of these fission products is governed largely by the rate at which they move through the soil to root surfaces.

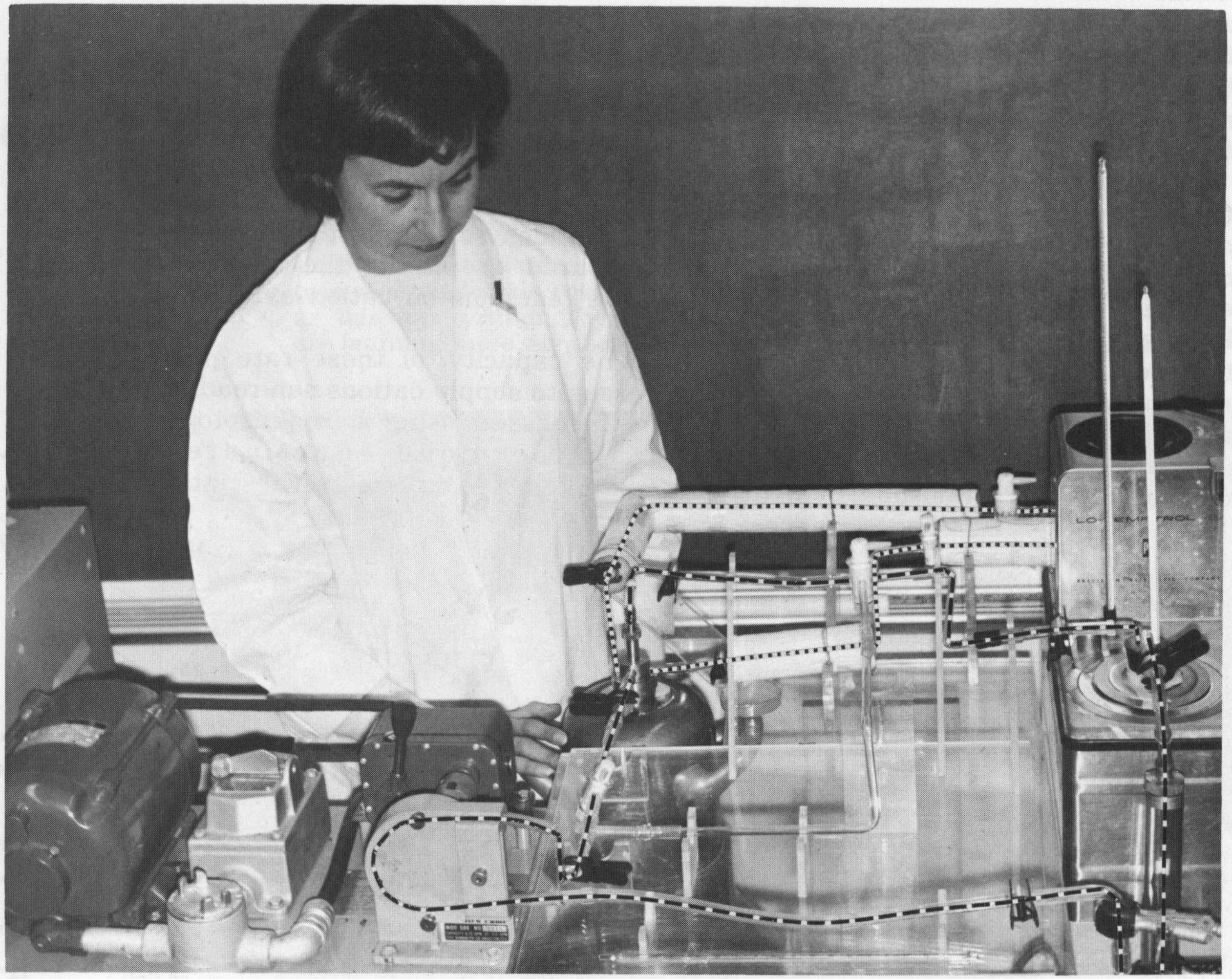
Investigations are being conducted to study cation retention properties of various clay minerals. Rates of cation release from these clays are evaluated in terms of our general knowledge of other rate processes involved in movement of cations to root surfaces to establish their relative importance in a given system.

If rates of cation release are fast compared to depletion due to plant uptake, they would affect cation availability by controlling equilibrium concentrations in the soil solution. On the other extreme, if cations are released at a much slower rate than depletion due to uptake processes, the retained cations would be unavailable for plant utilization in a given growing period, and the release of cations would be rate-limiting. It is necessary to establish which of these possibilities actually exist in the soil in order to know which soil properties must be measured to predict the effect of these reactions on cation availability.

The capacity of these rate processes to supply cations can readily be measured using a radioisotope tracer technique. An activated sodium iodide crystal with scintil-

VARIATION OF Na ACTIVITY COEFFICIENTS, gamma, and gamma', in Wyoming bentonite gels with varying clay and interstitial solution concentrations. Gamma and gamma' differ slightly in their definition.





CIRCULATION PATTERNS through an experimental apparatus used in making self-diffusion measurements in clay gels are shown by broken lines. A technician checks the operation.

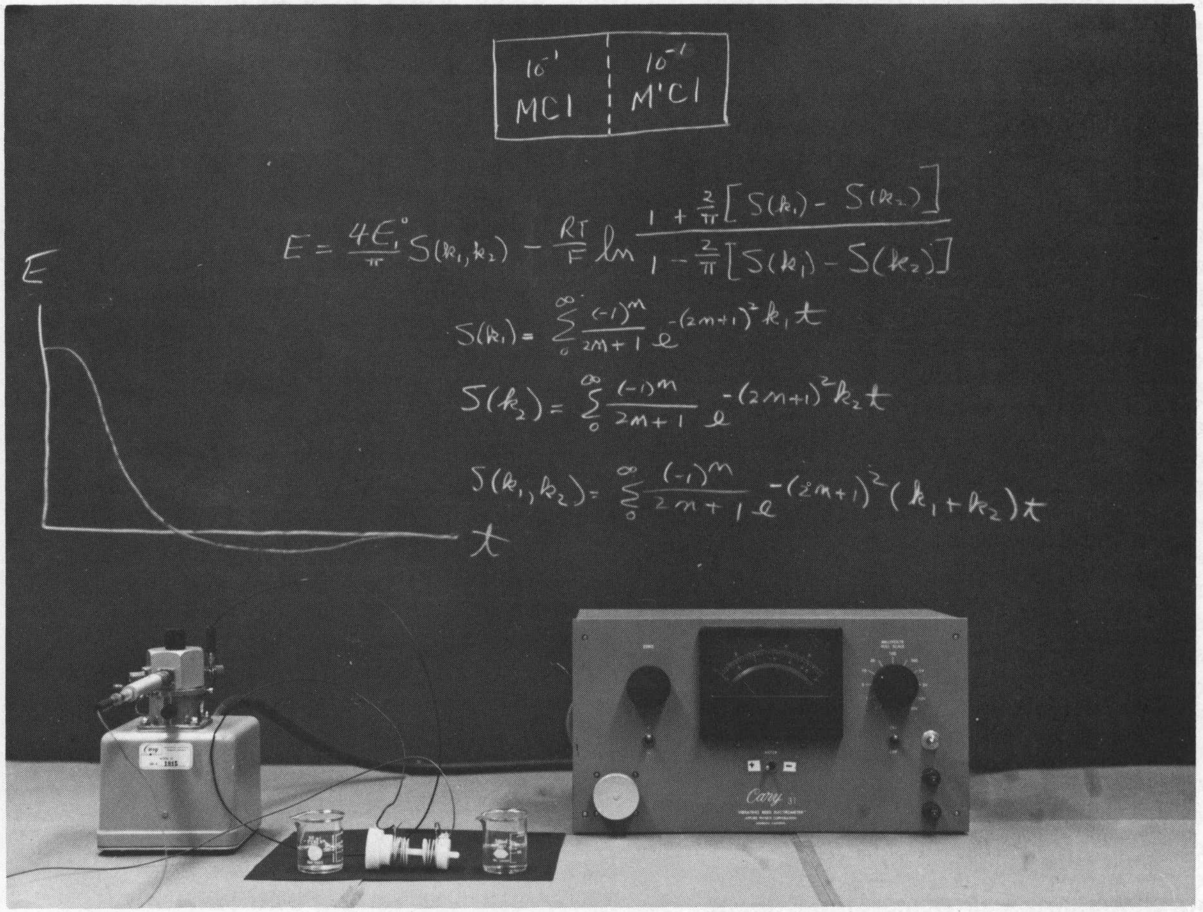
lation counter is particularly suited for measuring exchange reactions at low concentrations.

Cation diffusion rates (or ion exchange rates) in soils are functions of many properties of both the cation and the soil medium. These include, among others, cation size and valence, concentrations of free electrolyte, and the kind of clay. It would be difficult, if not impossible, to develop a physical model or molecular theory of diffusion to account for all these variables. However, cation diffusion rates in solution are well known from experimental measurements. We can then approach the simpler problem of investigating the reduction in diffusion rates in clays and soils compared to the rates in solution. Measurements of electrical conductance, thermodynamic activities and self-diffusion rates of cations and anions are being used to evaluate the relative effect of various properties of these systems in controlling reductions in diffusivity compared to aqueous solution. The final objective is to establish some fundamental property or independent measurement of the system from which diffusion coefficients can be predicted.

Counter-diffusion is one of the means by which fission products move through soils to plant roots. The present limiting diffusion laws (i.e., those which hold in idealized systems), and diffusion laws based upon consideration of a detailed physical model of systems to which they are applied, are not adequate when applied to heterogeneous soil systems.

Present investigations are concerned with developing a general theory of diffusion, that is, a theory which is applicable in non-ideal as well as ideal systems. A general theory has been postulated and is now being subjected to increasingly difficult tests. The basic premise of the theory is that a consideration of energy changes associated with diffusion will give rise to more general relationships than exist for material changes. This theory considers fluxes and gradients of energy rather than fluxes and gradients of molecules or ions.

Part of the experimental technique used to test the theory involves a measurement of the potential between two electrodes placed at the ends of resin membrane stacks. Prior to the start of an experiment, the membranes are divided into two stacks and each half equilibrated with different solutions of known composition. The half stacks are then brought together and the change in cell e.m.f. with time is continuously recorded and compared with the theoretical prediction of such change.



THIS LAB SETUP is one of the type used to measure change in cell e.m.f. with time. The equations on the board are of the form of those being tested.

RADIOBOTANY

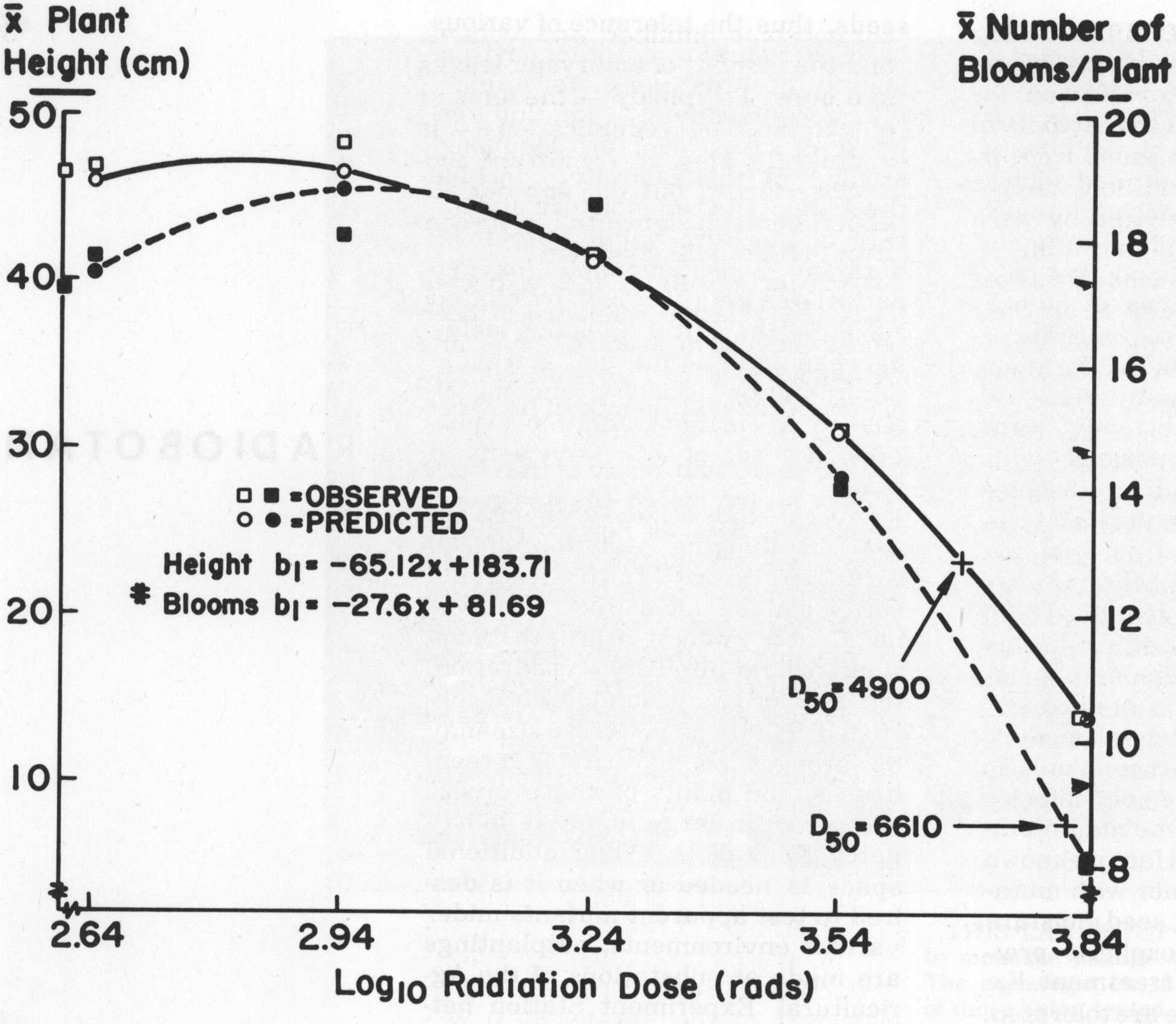
Research studies are generally concerned with biological effects, both genetic and physiological, of high-energy radiation on plant tissues. Emphasis is on higher plants of economic importance, and topics range from microchemical changes in subcellular organelles to gene frequency in field-scale populations.

In radiation studies of higher plants, the experimental material of choice in many cases is the dormant (seed) embryo, for many reasons: The embryo is in a steady state of "suspended animation," with metabolic functions at an absolute minimum, so the biological state is precisely repeatable from one experiment to another or from one year to another. Within a genetic strain, simply screening for seed size yields a reasonably homogeneous population having embryos of virtually identical degree of development. Seeds are usually rather small, so large numbers may be conveniently treated at one time. One may readily alter environmental factors known or suspected to interact with mutagenic treatment—e.g., seed moisture, oxygen tension, atmospheric pressure and fractionated treatment. Extremes of temperature are tolerated, and many aspects of measurement may be employed. Finally, all the food that sustains human life comes directly or ultimately from plants, many of which are reproduced by seeds, thus the tolerance of various seeds to radiation and other environmental hazards must be studied.

Factors modifying radiation response of seeds which are commonly studied at this laboratory include the amount of seed moisture and the rate of moisture vapor uptake by dormant seeds, amount of oxygen and storage period of irradiated seeds — the latter meaning delay between irradiation and germination, radiation intensity, and the effects of recurrent radiation exposures on seeds of successive generations.

Several kinds of measurements are used in studying radiation effects. Seedling growth from irradiated seeds can be measured, either as height or dry weight, in growth rooms wherein light intensity and duration, temperature, and humidity are controlled. Plants of moderate size may be brought to maturity in greenhouses, and plants of whatever size are grown in large numbers in irrigated field plots. When additional space is needed or when it is desired to test apparent mutants under various environments, outplantings are made at substations of the Agricultural Experiment Station network throughout Tennessee. Several kinds of radiations as well as chemical mutagens are being studied.

Laboratory measurements include microscopic assay of chromosomal



PREDICTING THE EFFECTS OF GAMMA irradiation on plant height (solid curve) of Hemerocallis fulva L. (common daylily), and mean number of blooms (broken line) on second-generation plants. Note the good agreement.

and developmental changes during germination of treated seeds. Microchemical variations are tested, for example, in mitochondrial activity in different embryonic tissues from irradiated seeds. In addition, "invisible" mutants are sought by such means as measuring variations in uptake of different ions by roots of plants having radiation in their ancestry, and by testing for enzyme or antienzyme production in seeds of advanced generations.

It has been known for many years that dormant seeds of different species vary in their sensitivity to ionizing radiation by 100-fold or more, but the reasons for such variation are only now emerging. The dormant embryos of many species have been examined microscopically to determine the developmental differences in embryonic organization among species. The relative importance of various embryonic factors to overall mutagen sensitivity forms an intricate pattern which is only partly revealed at present. In terms of gamma-ray sensitivity, the most important single genetic factor is apparently the size of cell nuclei in the region of the shoot growing point ("apical meristem"). Other embryonic factors contributing to the genetic control of radiosensitivity, in order of decreasing importance, are: volume of the terminal apex and number of cells therein, chromosome number and volume, and the number of embryonic leaves and buds. Polyploidy — the amount of chromosome reduplication — is apparently also an important controlling factor, but the appropriate experiments designed to test its contribution are still going on.

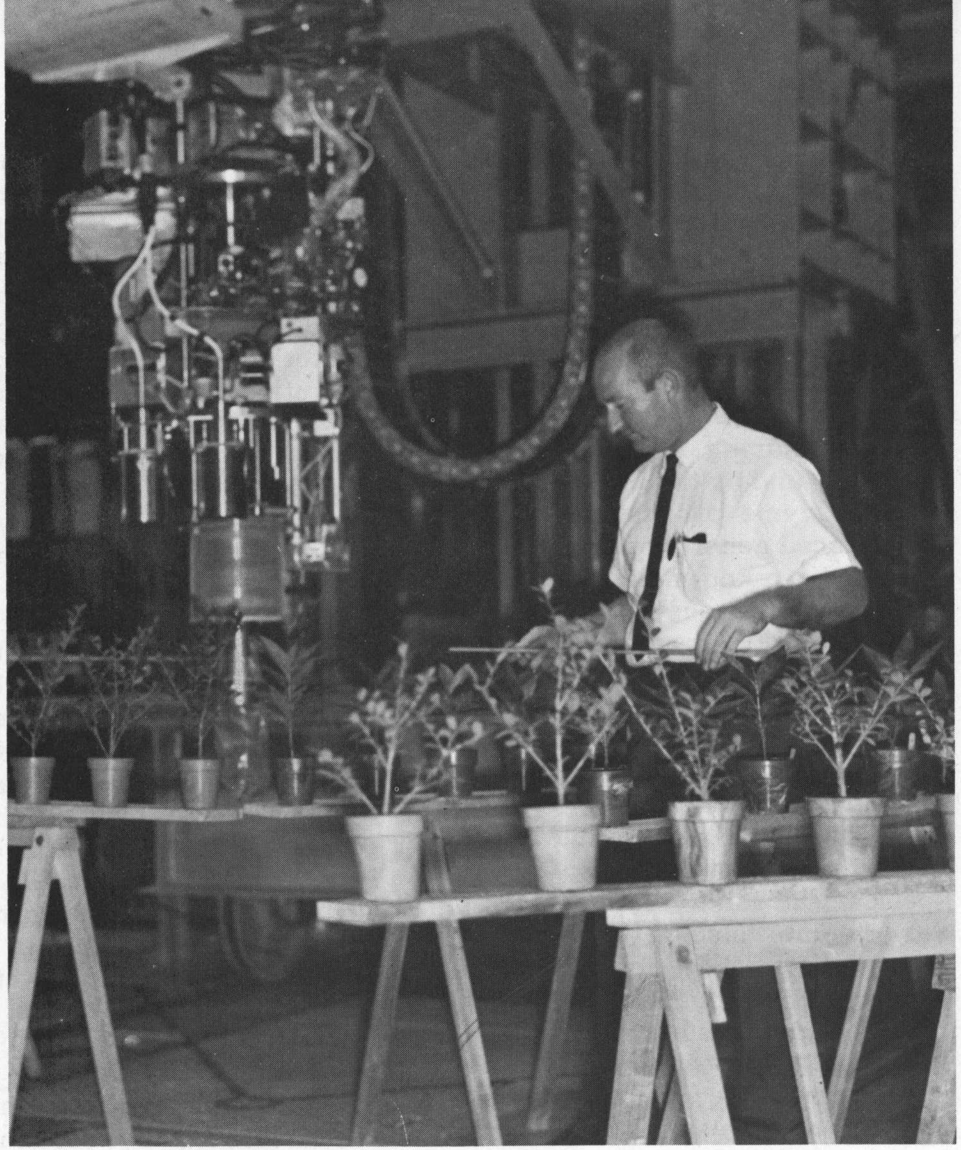
With further data, it is expected that the relative influence of each embryonic item toward governing response of seeds to neutrons and to chemical mutagens can be ascertained.

It appears that many of the effects shown by irradiated plants (growth reduction, delayed flowering, tumor formation, etc.) are the result of a disturbance in cellular metabolism. However, few explanations of a physiological nature have been offered in reference to specific morphological effects. Studies are being made, therefore, to correlate the physiological changes in cells with the abnormal or aberrant growth patterns of leaves, stems, roots, and flowers.

Such a study is being conducted to determine if radiation damage, as measured by seedling growth reduction, is related to a decreased efficiency of the physiological processes associated with germination and early seedling growth. Pea and okra seeds are irradiated with fast neutrons, and various parts of the germinating seedling (root, shoot, and cotyledons) are analyzed for mitochondrial activity and transformation of other biochemical substances. Results of the respiratory and chemical analyses are then correlated with growth of the plant parts at various time intervals after germination.

A study is being conducted to determine the sensitivity and mutation induction of horticultural plants after exposure to various sources of radiation. These plants are well adapted for this type of study because of their heterozygous nature (genetically unstable), and because desirable mutations or bud sports may be perpetuated by asexual reproduction. Information is made available to plant breeders concerning the nature and frequency of somatic mutations and the mechanisms which are responsible for radiation-induced changes in plant type.

Approximately 25 species have been exposed to fast neutrons and acute gamma radiation. Results of the response studies indicate that species may vary considerably in their tolerances to irradiation and can withstand much more gamma than fast neutron irradiation. As an example, chrysanthemum plants can survive a gamma-ray dose of approximately 6000 rads, whereas only 600 rads of fast neutrons may be lethal.



NEUTRON RADIATION experiments are made possibly by access to the Oak Ridge National Laboratory's health physics research reactor (HPRR). Here a scientist from the Agricultural Research Laboratory positions plants at varying distances from the reactor in order to vary the radiation dose received by each plant.

Radiation-induced mutations have been produced in several floricultural species (chrysanthemum, carnation, poinsettia, and Coleus) and in several woody ornamental species (holly, boxwood, and pyracantha). These mutations have been represented by changes in flower color, flower size, foliage color, and plant size. These results indicate that radiation may provide a valuable tool for the production of new horticultural varieties.

Dormant seeds of different species vary considerably in their response to ionizing radiation. Environmental factors including seed moisture, temperature, and atmospheric gases influence the sensitivity of seeds to ionizing radiation. When environment is controlled, seed radiosensitivity can be predicted on the basis of embryonic structural features. The question was asked: Is it possible to also predict seed sensitivity to unmoderated fission neutrons — a particulate type of radiation?

The Health Physics Research Reactor at Oak Ridge National Laboratory has been used to expose dormant seeds of various species. Seed sensitivity to fast neutrons is assessed on the basis of seedling dry weight per treatment. All seedlings are grown in environment-controlled growth rooms.

Growth response data show that species difference in sensitivity to fast neutrons is comparable to that observed with ionizing radiation. The pattern of growth reduction with increasing dose is also similar to that observed after gamma irradiation. However, in comparison, fast neutrons appear to be from 10 to 100 times more effective than gamma rays in causing growth reduction among the species tested. Indications are that environmental factors such as seed moisture and temperature do not modify the damage induced by fast neutrons.

Results to date do not permit a degree of accuracy in predicting seed sensitivity to fast neutrons comparable to that obtained for ionizing radiation. However, dosimetry of fast neutrons is influenced greatly by the amount of hydrogen, carbon, oxygen, and nitrogen in the seeds. With gamma irradiation the single most important embryonic feature associated with seed sensitivity is nuclear volume of apical initial cell (target size). Preliminary analysis of the fast neutron data indicates an apparent shift of emphasis to the number of apical initial cells (number of targets rather than size).

American Upland Cotton, **Gossypium hirsutum** L., is desirable agronomically and its high degree of adaptability permits production in all the leading cotton-growing countries of the world. Varieties of American Upland generally mature early and produce high yields of lint. The fiber of American Upland cotton is of medium quality. Sea Island cotton, **G. barbadense** L., is produced only in geographic areas with long growing seasons. However, Sea Island produces a high quality fiber — longer, stronger, and finer than American Upland. A stable hybrid variety possessing the desirable traits of both species has eluded cotton breeders and geneticists for over a century.

Hybridizing (crossing) the two species is easy, the F_1 hybrid is fertile and highly desirable from the standpoint of yield and fiber quality. The reproductive nature of cotton (primarily self-fertilized) prohibits the production of large quantities of F_1 seed for commercial production as is done so successfully in corn. It is necessary for the cotton breeder to continue selfing and selection for several generations in hopes of isolating stable lines. However, upon selfing the **hirsutum** X **barbadense** F_1 hybrid, a genetic breakdown or barrier occurs in the F_2 generation resulting in a predominance of inferior plant types; i.e., sterility, poor yield, low fiber quality.

Gamma irradiation of proembryos (1 to 4 cells) is being used to break chromosomes which may result in translocations. Translocations (exchanges of segments between chromosomes) can be used to transfer desirable traits from one species to another. If the technique is successful, it could overcome the genetic breakdown that has led to failure in the past.

Young hybrid bolls containing 30 to 40 proembryos are irradiated from 90 to 145 hours after pollination. A portable cobalt-60 source is used and doses range from 150 to 300 rads.

Some irradiated and non-irradiated hybrids have been grown through the F_3 generation. Data from fiber analyses indicate that population means are seldom altered by irradiation. However, population variances show decreases as well as increases in magnitude. A decrease in variance while maintaining high fiber quality is encouraging and is indicative of genetic stabilization.

A PORTABLE SOURCE is used in irradiation of cotton proembryos in an effort to hybridize American Upland and Sea Island varieties. The inset shows the stage of cellular development at which the irradiation is employed.

TRAINING AND EDUCATION

While the Laboratory has no specific committment in the area of training and education, its intimate connection with the College of Agriculture of the University of Tennessee makes it inevitable that this be included in the program. The Experiment Station is the research organization of the college and the Laboratory is one of the principal research units in the organization.

The research program of the Laboratory is coordinated with the needs of the University as well as those of the Atomic Energy Commission. Several types of assistantships are available through the Agricultural Experiment Station, and some of them involve participation in the Laboratory's research program. Under this system, the graduate student conducts research at the Laboratory during the hours not required for classroom instruction. Research done at the Laboratory is the student's thesis subject.

A member or members of the Laboratory's senior scientific staff serve as the major professor or on the graduate committee of the assistants and supervise their research and thesis preparation. The assistant is given as much independence as the scientist with whom he is working finds him capable of handling.

The Laboratory maintains a close working relationship with the Oak Ridge Institute of Nuclear Studies.* Research participants working under this program are supported financially by ORINS. These participants are associated with the Laboratory for varying periods of time.

There is also an association with other educational institutions through which the Laboratory's facilities may be made available to graduate students of these institutions. The student's institution provides his fee support and usually he completes all his course work there. Thesis research is done at the Laboratory on much the same basis as the work done by UT assistants. One or more senior staff members serve on the student's graduate committee, acting, in effect, as ex officio members of the faculty of the institution involved.

The Laboratory's program also allows for the accommodation of visitors who participate in the research efforts for various periods. Some of these visitors are faculty members of various institutions and are supported by their own institutions.

*Now Oak Ridge Associated Universities.

SKELETAL DEFECTS may be induced in prenatally irradiated animals of many species. A scientists, right, points out an example of such a defect to a research technician, left, and a graduate student.

Some are supported in part by their own institutions and in part by the Laboratory, and some are employed as temporary research participants by the Laboratory.

Graduate or undergraduate students are often employed to assist in research during the summer months.

Over the past several years the Laboratory has had an average of about eight to 14 graduate students and visiting research participants at any given time. Thesis and dissertation topics have covered a wide range of subjects, but most have dealt with some phase of radiation research with large animals and plants.